



Research article

Nitrous oxide and methane production from denitrifying woodchip bioreactors at three hydraulic residence times

Morgan P. Davis^{a,*}, Emily A. Martin^b, Thomas B. Moorman^c, Thomas M. Isenhardt^d, Michelle L. Soupir^b^a Dep. of Agronomy, Iowa State Univ., 2104 Agronomy Hall, Ames, IA, 50011, USA^b Dep. of Agricultural and Biosystems Engineering, Iowa State Univ., 1340 Elings Hall, Ames, IA, 50011, USA^c National Laboratory for Agriculture and the Environment, USDA-ARS, 1015 N. University Blvd., Ames, IA, 50011, USA^d Dep. of Natural Resource Ecology and Management, Iowa State Univ., 339 Science Hall II, USA

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ABSTRACT

Denitrifying bioreactors remove nitrate (NO_3^-) from agricultural drainage and are slated to be an integral part of nitrogen reduction strategies in the Mississippi River Basin. However, incomplete denitrification can result in nitrous oxide (N_2O) production and anaerobic conditions within bioreactors may be conducive to methane (CH_4) production via methanogenesis. Greenhouse gas production has the potential to trade excess NO_3^- in surface water with excess greenhouse gases in the atmosphere. Our study examined N_2O and CH_4 production from pilot scale (6.38 m^3) bioreactors across three hydraulic residence times (HRTs), 2, 8, and 16 h. Production was measured from both the surface of the bioreactors and dissolved in the bioreactor effluent. Nitrous oxide and CH_4 was produced across all HRTs, with the majority dissolved in the effluent. Nitrous oxide production was significantly greater ($P < 0.05$) from 2 h HRTs ($478.43 \text{ mg N}_2\text{O m}^{-3} \text{ day}^{-1}$) than from 8 ($29.95 \text{ mg N}_2\text{O m}^{-3} \text{ day}^{-1}$) and 16 ($36.61 \text{ mg N}_2\text{O m}^{-3} \text{ day}^{-1}$) hour HRTs. Methane production was significantly less ($P < 0.05$) from 2 h HRTs ($0.51 \text{ g C m}^3 \text{ day}^{-1}$) compared to 8 ($1.50 \text{ g C m}^3 \text{ day}^{-1}$) and 16 ($1.69 \text{ g C m}^3 \text{ day}^{-1}$) hour HRTs. The 2 h HRTs had significantly greater ($P = 0.05$) impacts to climate change compared to 8 and 16 h HRTs. Results from this study suggest managing HRTs between 6 and 8 h in field bioreactors could minimize total greenhouse gas production and maximize NO_3^- removal.

1. Introduction

Nitrate (NO_3^-) losses from agriculture in the upper Midwest of the United States contribute to the extent of the hypoxic zone in the Gulf of Mexico (David et al., 2010) and lead to high NO_3^- concentrations in surface drinking water sources (Schilling and Zhang, 2004). Mid-western states have responded to environmental concerns of nitrogen (N) loss through the implementation of nutrient reduction strategies. Nutrient reduction strategies utilize both in-field and edge of field practices to reduce NO_3^- losses from predominantly corn (*Zea mays* L.) and soybean [*Glycine max* (L.) Merr.] agriculture (IDALS, 2017; Illinois EPA, 2015). Edge of field practices including wetlands, saturated buffers, and bioreactors have shown promise to reduce NO_3^- losses from artificial subsurface, tile, drainage (Addy et al., 2016; Groh et al., 2015; Jaynes and Isenhardt, 2014; Tomer et al., 2013). Bioreactors are an integral part of nutrient reduction strategies and comprise up to 18% of planned reductions within some scenarios of the Iowa Nutrient

Reduction Strategy. Bioreactor removal efficiency is dependent on several factors, including the hydraulic residence time (HRT) of tile water being treated. Addy et al. (2016) conducted a meta-analysis and found significantly less mass NO_3^- removal in bioreactors with HRTs less than 6 h. For maximum NO_3^- removal, HRT should be included in bioreactor design to optimize NO_3^- removal.

Bioreactors create anaerobic conditions ideal for denitrification and other forms of anaerobic respiration (Christianson et al., 2009; Jaynes et al., 2008; Schipper et al., 2010). Incomplete denitrification in bioreactors can result in nitrous oxide (N_2O) production (Elgood et al., 2010; Greenan et al., 2009; Warneke et al., 2011). Furthermore, bioreactors can be sources of methane (CH_4) production via methanogenesis (Elgood et al., 2010; Healy et al., 2012). Nitrous oxide and CH_4 are the second and third largest contributors to global radiative forcing respectively. Nitrous oxide and CH_4 production have been observed from bioreactors, but measurements do not always include both emissions dissolved in treated water and from bioreactor surfaces (David

* Corresponding author.

E-mail address: morgand@iastate.edu (M.P. Davis).<https://doi.org/10.1016/j.jenvman.2019.04.055>

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et al., 2016; Woli et al., 2010). Laboratory studies have examined N_2O and CH_4 production at different HRTs (Greenan et al., 2009; Healy et al., 2012; Bock et al., 2018), but these studies were conducted on relatively small columns less than 0.001 m^3 . Recent bioreactor studies have also highlighted a potential role of bioreactors to reduce N_2O emissions through NO_3^- removal, reducing the potential downstream denitrification of NO_3^- (Moorman et al., 2010). The Intergovernmental Panel on Climate Change's (IPCC) defines downstream emissions as indirect emissions and includes groundwater, rivers, and estuaries as potential sources. If N_2O and CH_4 production is greater than reductions to indirect N_2O emissions, bioreactors may pollution swap NO_3^- in surface waters with increased greenhouse gas emissions.

This study utilized nine pilot scale (6.38 m^3) bioreactors to maintain tile water HRTs of 2, 8, and 16 h. Our study objectives were to (i) determine the effect of HRT on N_2O and CH_4 production from both soil cover and effluent, (ii) examine potential correlations of dissolved oxygen (O_2) with N_2O and CH_4 production, and (iii) determine the effect of HRT on climate change.

2. Materials and methods

2.1. Bioreactor design and operation

Greenhouse gas and water chemistry measurements were taken from nine pilot scale ($5.8 \times 1.0 \times 1.1 \text{ m}$) bioreactors designed specifically for research at Iowa State University's Agronomy and Agricultural Engineering Research Farm located west of Ames, IA ($42^\circ 01' 01'' \text{N}$, $93^\circ 46' 48'' \text{W}$). Bioreactors were installed in September 2014 and only active for three weeks in 2015 prior to this study. Local hardwood woodchips, supplier details in Christianson et al. (2010), were housed in concrete trenches capped with 20 cm of excavated soil. The site was seeded to a Midwestern wildflower mix in fall of 2015. Bioreactor influent water was sourced from a 30.5 cm drainage tile that passes through the University farm. A brass gate-valve controls inlet flows into each bioreactor. Water height in the bioreactors is set by water control structures (Agri Drain, Adair, IA) at each outlet. Water was held to 1 m above the bottom of each bioreactor for a total saturated volume of 6.38 m^3 per bioreactor. Each bioreactor is equipped with two sampling wells (10.2 cm diameter) located 1.42 m and 4.26 m from the inlet (Fig. 1). Sampling wells were fully screened (1 m) to the height of the water in the bioreactors. Design specifics and operation of the pilot-scale bioreactors can be found in Hoover et al. (2017).

Flow rates were calculated for three HRTs, 2, 8, and 16 h, using saturated volume and media porosity. A potassium bromide tracer study was conducted to determine media porosity (Hoover et al., 2017). Hydraulic residence times were assigned in a randomized complete block design. Bioreactors were blocked by water storage tank source, and HRT treatments were randomly assigned within each block, for a total of nine bioreactors (Fig. 1). As part of a companion study (Martin et al., 2010) examining NO_3^- removal and flow dynamics, flow rates were found to be significantly different from one another (data not shown).

2.2. Sample collection

Samples were collected simultaneously on a weekly basis from August 15 through October 26 in 2016 and from June 1 through July 6 in 2017. Bioreactors were not drained throughout 2016–2017 winter months. Flow was returned to the bioreactors in late May of 2017 and one week of flow was allowed before collection began on Jun 1, 2017. Water that over-winters in bioreactors has the potential to contain elevated concentrations of dissolved N_2O and CH_4 and could confound our objective to determine the effect of HRTs on greenhouse gas production. Measurements were limited in 2017 due to abnormally low precipitation. The source tile supplying drainage water to the bioreactors stopped flowing, inhibiting our ability to maintain desired

HRTs. Sample collection took place for 78 days in 2016 and 36 days in 2017.

Samples were collected between 10:00 a.m. to 2:00 p.m. to limit potential diurnal variation in greenhouse gas emissions from the soil surfaces (Parkin and Venterea, 2010). A typical sample collection day consisted of aqueous sample collection from the inlet, sampling wells, and outlet of each bioreactor. Sampling wells were evacuated using a peristaltic pump prior to sample collection. A 125 ml sample was then collected for NO_3^- . Samples were acidified, stored on ice in the field, and at 4°C in the laboratory until analysis. Next, dissolved greenhouse gas samples were collected into 10 ml syringes. A syringe was held onto the end of the sampling house of the pump and filled, limiting gas loss through atmospheric exposure. Samples (10 ml) were then injected into evacuated 20 ml glass vials sealed with butyl rubber stoppers (Voigt Global). Glass vials contained 0.3 ml of 80% zinc chloride to preserve dissolved gas concentrations until analysis. Duplicate samples were taken from each sampling point. After aqueous sample collection, dissolved O_2 and temperature were measured using a YSI Pro ODO field probe (YSI Inc.). The dissolved O_2 probe was carefully lowered into each sampling structure and allowed to equilibrate before measurements were recorded.

Greenhouse gas samples from bioreactor surfaces were collected concurrently with aqueous sampling from circular static vented chambers equipped with automated sampling equipment (Davis et al., 2018). Schedule 40 PVC anchors (30 cm diameter, 15 cm tall) were installed 2.03 and 4.86 m from the inlet of each bioreactor (Fig. 1). Chamber tops were constructed from 30 cm sections of schedule 40 PVC to a height of 15 cm and are described in Parkin and Venterea (2010). Chamber tops were vented to maintain atmospheric pressure and covered with reflective tape to limit solar radiation absorbance. Chambers were placed on anchors and automated samplers attached to a butyl rubber stopper sampling port located on the top of each chamber. Automated samplers collected gas samples at time 0, 15, 30, and 45 min. Samples were collected into 20 ml syringes equipped with stopcocks. After sample collection stopcocks were closed until laboratory analysis. Air temperature was measured inside each static chamber using a HOBO temperature pendant (Onset Computer Corp.).

2.3. Sample analysis

Nitrate was analyzed on a Seal Analytical (Mequon, WI) AQ2 discrete autoanalyzer (AQ2 method EPA-114-A, Rev. 7). Samples are measured as $\text{NO}_3\text{-N} + \text{NO}_2\text{-N}$. Nitrate is reduced by copperized cadmium to NO_2 and measured spectrophotometrically at 520 nm. The method detection limit is 0.03 mg N L^{-1} .

Surface gas samples were prepared for analysis in the laboratory by injecting 13 ml of sample into 6 ml vials sealed with butyl rubber stoppers. Dissolved gas samples were prepared for analysis in the laboratory by first adjusting to atmospheric pressure and then overfilled with 7 ml of helium. Samples were shaken for 15 min on a reciprocal shaker to equilibrate N_2O and CH_4 concentration with the vial headspace.

Greenhouse gas concentrations were measured on a gas chromatograph (GC) (SRI instruments, model 8610). An automated sampler (Arnold et al., 2001) introduced gas samples into the inlet valve of the GC where they traveled through a stainless steel column (0.3175 cm diameter $\times$ 74.54 cm long) packed with Haysep D to a ^{63}Ni electron capture detector (N_2O) and then a flame ionization detector (CH_4). Sample concentrations were calculated using linear regression coefficients of N_2O and CH_4 standards (Air Liquide Specialty Gases) and the universal gas law. Total gas content in dissolved samples was calculated using Henry's law and Bunsen absorption coefficients. Greenhouse gas surface fluxes were calculated using the HMR package in R v3.1.2 (The R Foundation, 2014). The HMR model was attempted first for each set of time series samples. If the HMR model failed to produce a flux, the software used linear regression or assigned a "no flux" value of zero

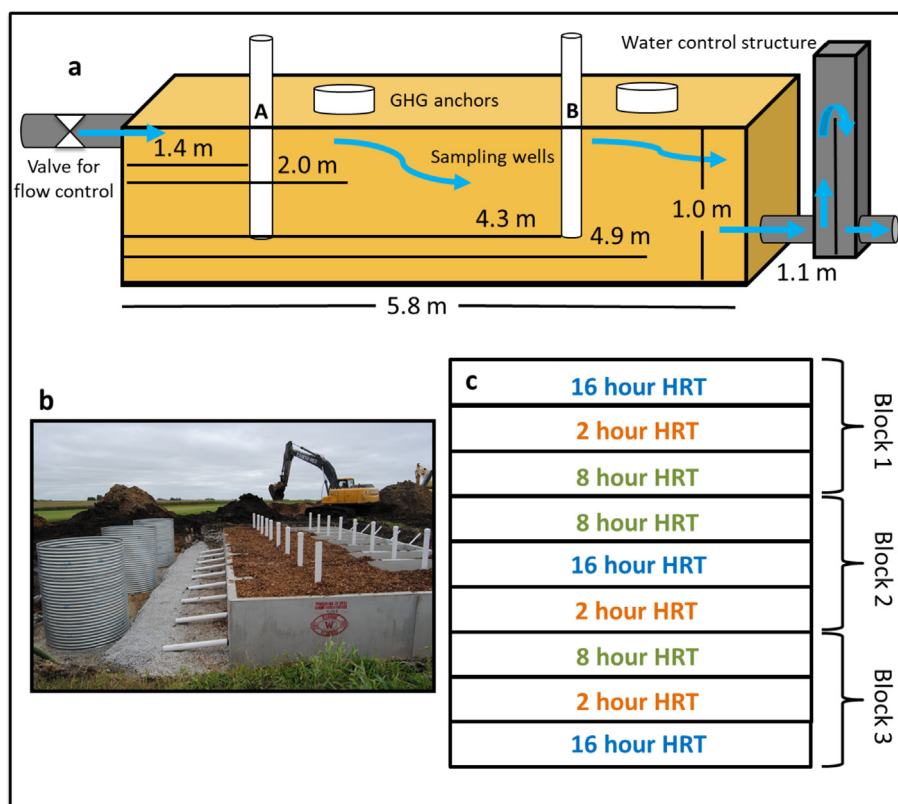


Fig. 1. (a) Bioreactor layout with sampling locations, distances from the inlet, and illustrated flow path. (b) Photo of installation before control structures were installed at the outlet. (c) Randomized complete block design for hydraulic residence times (HRTs) of pilot scale bioreactors. Bioreactors were blocked by inlet pipe.

(Pedersen et al., 2010). Cumulative emissions were calculated by linearly interpolating between sampling dates and summing daily emission rates for the study period.

2.4. Greenhouse gas production and statistical analysis

Mass N_2O and CH_4 production was calculated for each bioreactor by subtracting inlet mass load from surface and dissolved outlet mass loads. Cumulative surface emissions were calculated by multiplying cumulative emissions for the study period by the surface area (6.38 m^2) of each reactor. Dissolved concentrations and flow rates were used to calculate mass loads of dissolved gases for each reactor. Production rates ($\text{g m}^{-3} \text{ day}^{-1}$) were calculated within the bioreactors at well sampling points and the outlet. Indirect emissions were calculated using the IPCC's emissions factor for estimating N_2O losses from leached water of $0.0075 \text{ kg N}_2\text{O-N kg}^{-1} \text{ NO}_3^- \text{-N}$ leached. The indirect emissions factor is comprised of three equal emissions factors for groundwater, rivers, and estuaries at $0.0025 \text{ kg N}_2\text{O-N kg}^{-1} \text{ NO}_3^- \text{-N}$ leached (Intergovernmental Panel on Climate Change, 2006). In this study the groundwater emissions factor was directly measured as dissolved N_2O entering the reactors and an emissions factor of $0.005 \text{ kg N}_2\text{O-N kg}^{-1} \text{ NO}_3^- \text{-N}$ was used to estimate emissions from rivers and estuaries.

2.5. Impact on climate change

To compare the radiative forcing potential from each bioreactor gas production rates were converted to carbon dioxide equivalents (CO_2e). Global warming potentials (GWP) of CH_4 and N_2O are 28 and 265 times the potential of CO_2 , respectively, for a 100 year adjustment not including climate carbon feedbacks (Myhre et al., 2013). Carbon dioxide equivalents were calculated by multiplying production rates by respective GWP values.

2.6. Statistical analysis

Statistical analyses were conducted in R v3.1.2. Greenhouse gas production rates and climate change impacts were compared using a repeated measures two-factor analysis of variance with a Tukey's honest significance post-hoc test. Dissolved gas concentrations were compared to water chemistry parameters using linear regression models. A quadratic model was used to estimate minimum CO_2e to NO_3^- removed ratios.

3. Results and discussion

3.1. Nitrate removal

Nitrate was removed from the source water within all nine bioreactors. Nitrate concentrations were reduced significantly ($P < 0.05$) from the inlet to the outlet in all three HRTs. Mass NO_3^- load removal increased significantly ($P = 0.05$) with decreasing HRT, 6.70 kg-N (2 h), 5.97 kg-N (8 h), and 5.14 kg-N (16 h) (Table 1). Additional details on NO_3^- removal dynamics and dissimilatory NO_3^- reduction to ammonium can be found in Martin et al. (2010).

3.2. Nitrous oxide production

Cumulative N_2O emissions from the surface and outlet were greater than inlet contributions across all nine bioreactors, resulting in net production of N_2O (Table 1). Inlet tile N_2O concentrations showed little variation over the study period and ranged from 12.0 to $36.2 \mu\text{g N}_2\text{O-N L}^{-1}$, similar to other tile samples collected within Central Iowa (Parkin et al., 2016). Tile inlet loads were 1.06 (2hr HRT), 0.27 (8hr HRT), and 0.17 (16 h HRT) $\text{g N}_2\text{O-N day}^{-1}$. Dissolved N_2O loads leaving the bioreactor outlets were 4.17 (2hr HRT), 0.46 (4hr HRT), and 0.41 (16 h HRT) $\text{g N}_2\text{O-N day}^{-1}$. Dissolved N_2O loads from the 2 h HRT treatment

Table 1

Nitrate (NO_3^-) removal and nitrous oxide (N_2O) production from bioreactors at three different hydraulic residence times (HRTs). Nitrate removal is presented in load (kg-N) for the study period. Lowercase letters indicate significant differences among dissolved loads. Loads with different letters denote significant difference at p -values < 0.05 . Uppercase letters indicate differences among HRT production rates, different letters denoting significance as p -value < 0.05 . Surface fluxes were not significantly different among HRTs. Nitrous oxide production loads were used to calculate percentages of kg $\text{N}_2\text{O-N}$ produced per kg NO_3^- -N removed.

HRT	NO ₃ ⁻ mass removal kg-N	N ₂ O mass loads			N ₂ O production mg N ₂ O-N m ⁻³ day ⁻¹	kg N ₂ O-N kg ⁻¹ NO ₃ -N removed %
		Surface	Dissolved			
			Inlet	Outlet		
2 h	6.70	0.004	1.06	4.17	478.43 A	5.19
8 h	5.97	0.005	0.27	0.46	28.95 B	0.35
16 h	4.14	0.002	0.17	0.41	36.61 B	0.52

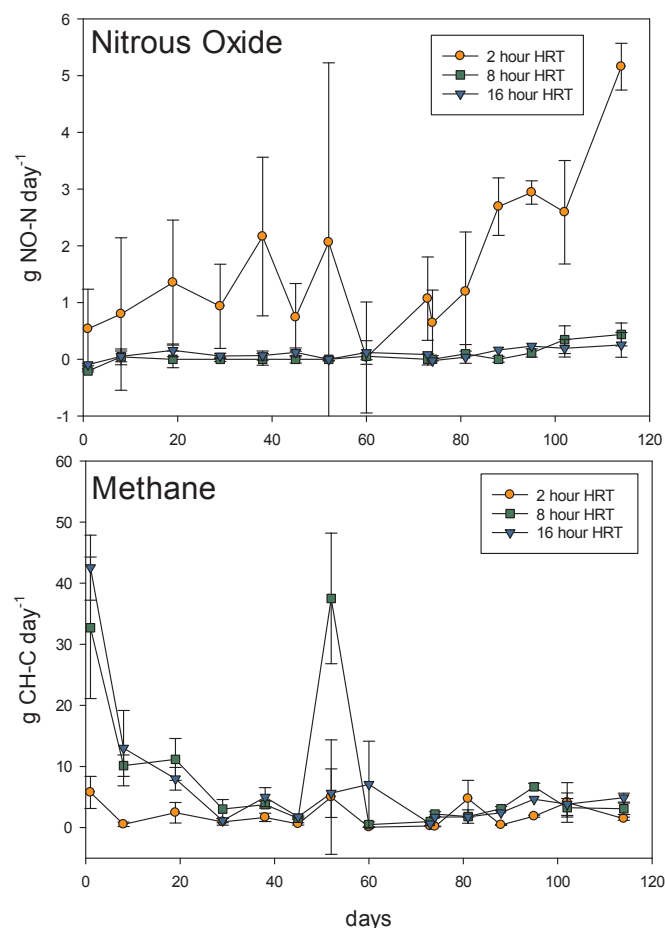


Fig. 2. Nitrous oxide (N_2O) and methane (CH_4) production over 114 days from both 2016 and 2017. Time was not a significant ($P < 0.05$) factor in N_2O production, but was significant for CH_4 in a two factor repeated measures analysis of variance.

were significantly greater ($P < 0.05$) than both 8 and 16 h HRTs. Nitrous oxide flux from the surface of the bioreactors were much lower than dissolved counterparts, emitting 0.004 (2hr HRT), 0.005 (8hr HRT), and 0.002 (16hr HRT) g $\text{N}_2\text{O-N}$ day. Surface N_2O emissions were not significantly different from one another ($P = 0.40$) across the three HRTs (Table 1).

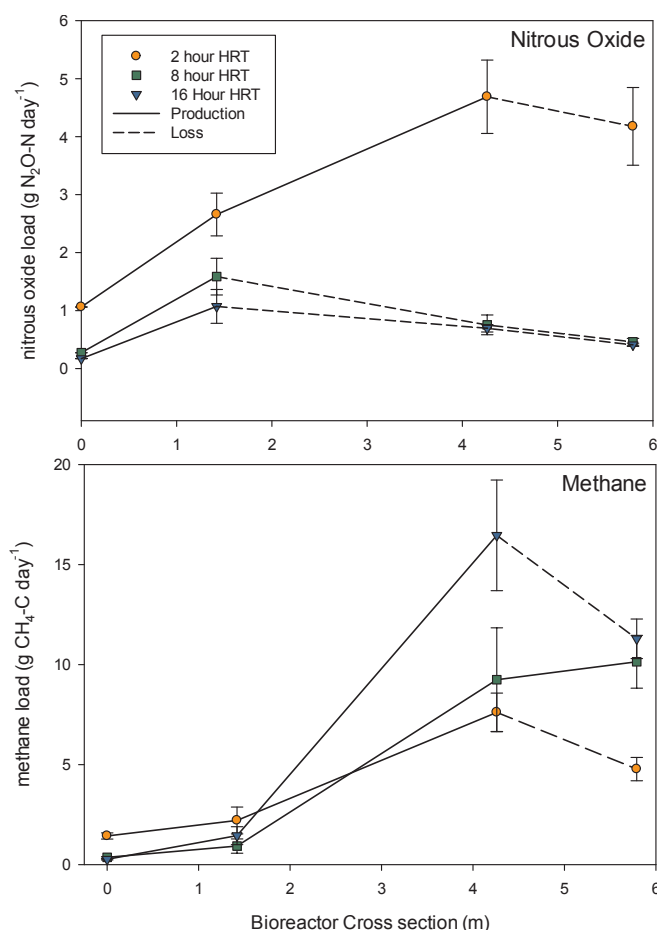


Fig. 3. Nitrous oxide (N_2O) and methane (CH_4) loads, including dissolved and surface emissions at sampling points through the bioreactors. Production are positive slopes between points. Losses were negative slopes between points. Losses were not significant at any hydraulic residence times (HRTs) for both gases. Nitrous oxide production was greatest between the bioreactor inlet (0 m) and well A (1.42 m). Methane production was greatest between the bioreactor well A (1.42 m) and well B (4.26 m).

Nitrous oxide production did not change significantly over time ($P < 0.05$) (Fig. 2). The 2 h HRT bioreactors produced the most N_2O on average, 478.43 mg $\text{N}_2\text{O-N m}^{-3} \text{ day}^{-1}$, significantly greater ($P < 0.05$) than the 8 h HRT (28.95 mg $\text{N}_2\text{O m}^{-3} \text{ day}^{-1}$) and 16 h HRT (36.61 mg $\text{N}_2\text{O m}^{-3} \text{ day}^{-1}$) N_2O production. Production from the 8 h HRT and 16 h HRT were not significantly different from one another ($P = 0.98$). Our results agree with Bock et al. (2108) who found greater N_2O loss from column bioreactors (0.007 m^3) with a 3 h HRT compare to 6 and 12 h HRT, but differ from the laboratory study by Greenan et al. (2009) that found no significant difference in N_2O production between hydraulic residence times ranging from 1.5 to 6 days. Nitrous oxide emissions within 2 h HRTs were similar to Warneke et al. (2011), who found emissions of 380 mg $\text{N}_2\text{O-N m}^{-3} \text{ day}^{-1}$ over summer months from a bioreactor with a 6 day HRT.

Surface fluxes represented 0.1% (2hr HRT), 2.6% (8hr HRT), and 0.8% (16hr HRT) of total N_2O production. Surface N_2O fluxes were similar to other bioreactor studies using static chambers (David et al., 2016; Warneke et al., 2011; Woli et al., 2010). However, these studies were conducted on bioreactors without a soil cap and anchors were placed directly into the woodchips. In our study, N_2O emissions from the bioreactor surface could include N_2O from denitrification within the soil cap. Christianson et al. (2013) suggested N_2O emissions could be mitigated through soil cap implementation. Surface emissions from our study with a soil cap were similar to emissions found in Warneke et al.

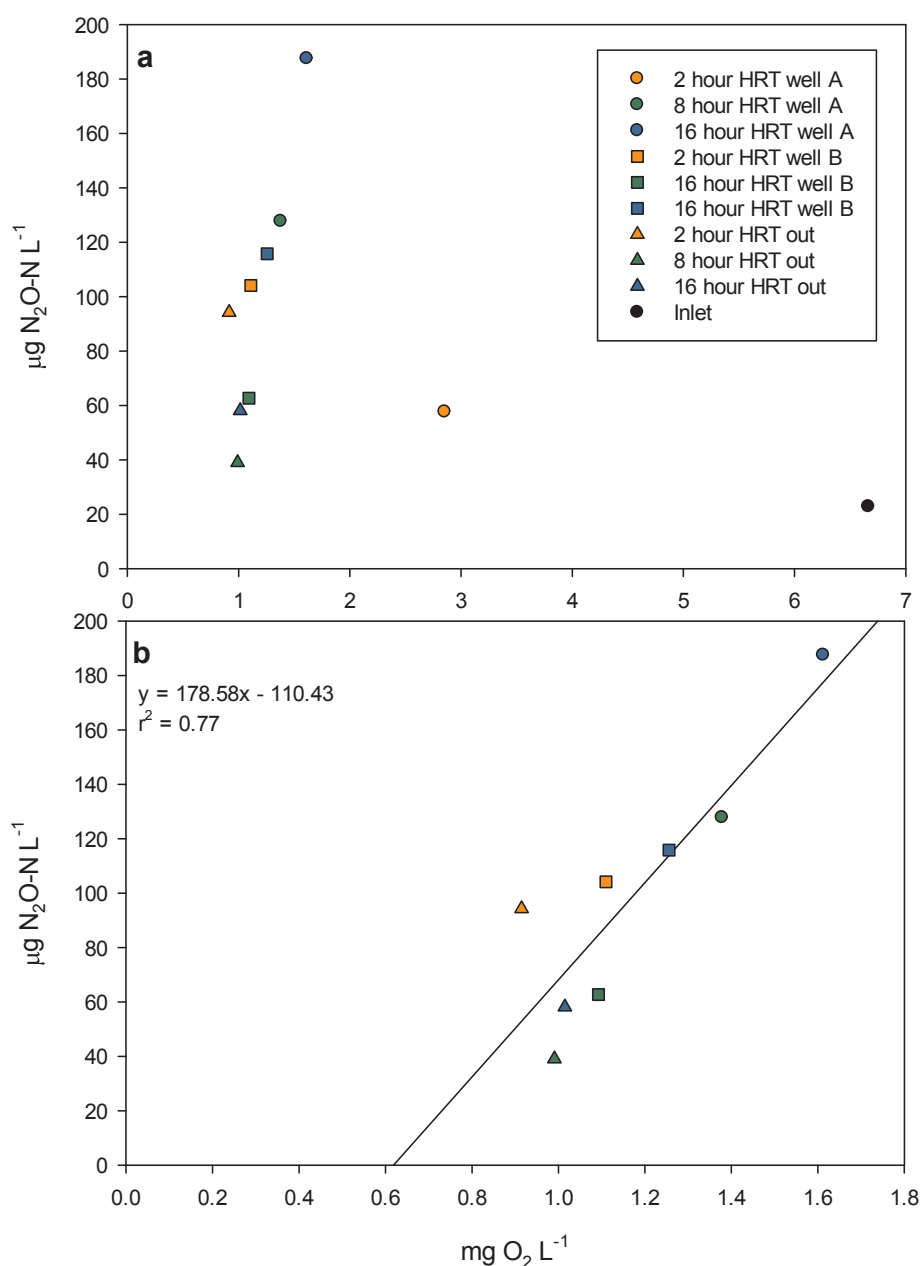


Fig. 4. (a) Average dissolved nitrous oxide (N_2O) concentrations and average dissolved oxygen (O_2) for all bioreactors and positions. (b) Average dissolved N_2O concentrations and average dissolved O_2 below $2 \text{ mg O}_2 \text{ L}^{-1}$ with linear regression analysis.

Table 2

Methane (CH_4) and Oxygen (O_2) mass loads and production. Lowercase letters indicate significant differences among dissolved loads. Loads with different letters denote significant difference at p -values < 0.05 . Uppercase letters indicate differences among HRT production rates, different letters denoting significance as p -value < 0.05 . Surface fluxes were not significantly different among HRTs.

HRTs	O_2 mass loads	CH ₄ mass loads		CH ₄ production	
		Surface	Dissolved	Inlet	Outlet
	Inlet				
	$\text{g O}_2 \text{ day}^{-1}$	$\text{g CH}_4\text{-C day}^{-1}$			$\text{g CH}_4\text{-C m}^{-3} \text{ day}^{-1}$
2 h	311.2	0.18	1.43	4.60	0.51 B
8 h	80.0	0.31	0.36	9.83	1.50 A
16 h	45.3	0.86	0.26	10.43	1.69 A

(2011) and greater than emissions found in David et al. (2016) and Woli et al. (2010), all without soil caps. Surface N_2O fluxes from the bioreactors were less than fluxes associated with N fertilized corn in summer months and similar to fluxes from perennial plant systems on a per ha basis (Iqbal et al., 2015; Parkin and Kaspar, 2006). The majority of N_2O produced exited the bioreactors through the outlet as dissolved N_2O .

Dissolved N_2O production within bioreactors was greatest from the inlet to well A across all HRTs (Fig. 3), indicating the greatest rate of N_2O production was in the first 1.42 m of treatment. Production continued in the 2 h HRT from well A to B, but was not observed in 8 and 16 h HRTs. Nitrous oxide inlet load was significantly ($P < 0.05$) greater than the outlet load within the 2 h HRT, but not within the 8 or 16 h HRTs. Overall N_2O concentrations were not correlated with NO_3^- concentrations ($r^2 = 0.003$) or dissolved O_2 ($r^2 = 0.002$) (Data not shown). However, when comparing average dissolved O_2 and N_2O concentrations by bioreactor position, we observed a correlation

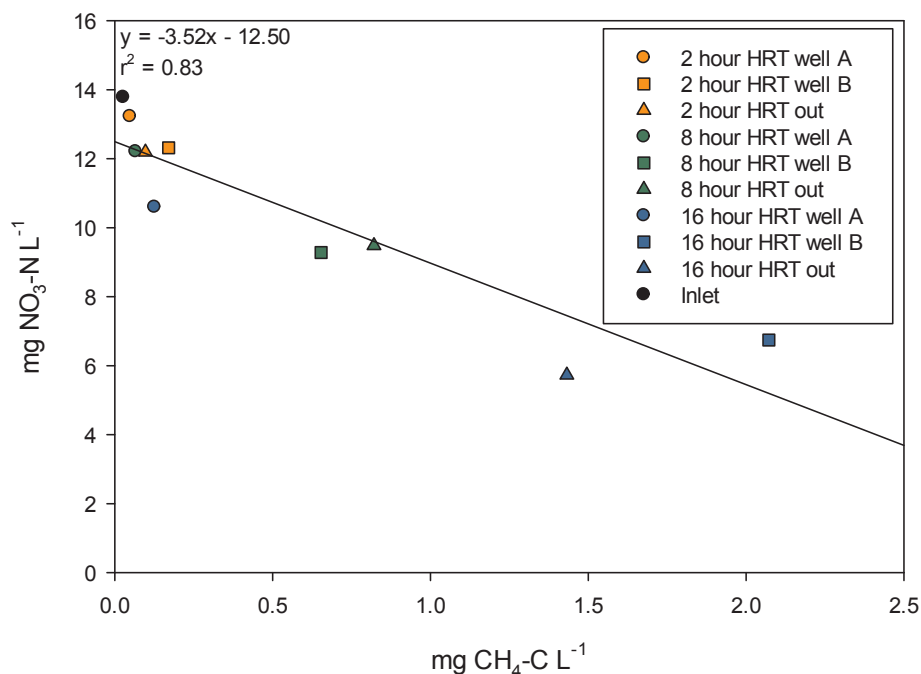


Fig. 5. Average dissolved methane (CH_4) concentrations and average dissolved nitrate (NO_3^-) for all reactors and positions.

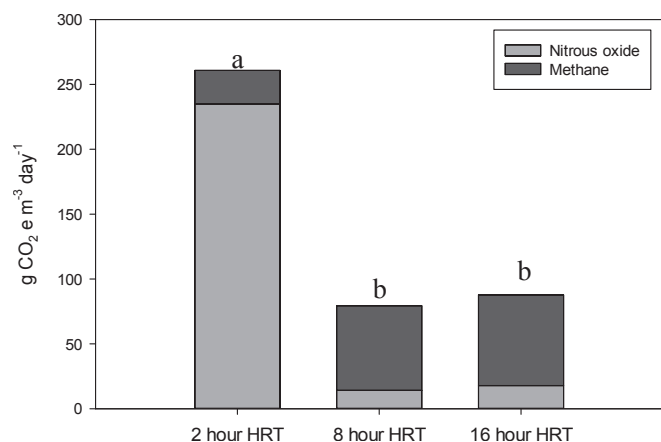


Fig. 6. Impacts on climate change in Carbon dioxide equivalents (CO_2e) of three hydraulic residence times (HRTs). Letters denote significant differences at p -values < 0.05 .

between decreasing average N_2O concentration with decreasing dissolved O_2 concentrations below $2 \text{ mg O}_2 \text{ L}^{-1}$ ($r^2 = 0.77$) (Fig. 4). The inlet and well A of the 2 h HRT averaged above $2 \text{ mg O}_2 \text{ L}^{-1}$ and did not follow a similar trend. In this case N_2O production was the highest observed, despite dissolved O_2 concentrations typically above $2 \text{ mg O}_2 \text{ L}^{-1}$. This could be explained by the fact that dissolved O_2 measurements were taken from water flowing through the woodchips in the reactor, while N_2O production likely took place at locally anaerobic sites on and within woodchips (Moorman et al., 2010).

Overall, N_2O emissions were 5.19% of the total NO_3^- removed at 2 h HRT and only 0.38% for the 8 h HRT and 0.50% for the 16 h HRT. The 2 h HRT percentage was greater than the range observed in the literature of < 1 –4.7% (Christianson et al., 2013; David et al., 2016; Warneke et al., 2011; Woli et al., 2010). However, these studies did not all include dissolved loads that proved to represent the majority of N_2O loss in this study. If N_2O produced from NO_3^- removal is less than estimated N_2O production from rivers and estuaries downstream, bioreactors would remove NO_3^- while reducing indirect N_2O emissions. In this study the 8 h HRT was the only treatment that removed

NO_3^- and reduced potential indirect N_2O emissions. The difference of bioreactor emissions from pre-treated emissions, including indirect emissions, were $2.84 \text{ g N}_2\text{O day}^{-1}$ (2 h HRT), $-0.06 \text{ g N}_2\text{O day}^{-1}$ (8 h HRT), and $0.01 \text{ g N}_2\text{O day}^{-1}$ (16 h HRT). Furthermore, N_2O emissions after bioreactor treatment were not significantly different (Welch two sample t-test) from pre-treated water for the 2 h ($P = 0.11$), 8 h ($P = 0.51$), and 16 h ($P = 0.76$) HRTs. These results support other studies concluding bioreactors are not swapping NO_3^- removal in surface waters with increased N_2O in the atmosphere.

3.3. Methane production

Bioreactors were a source of CH_4 production across all HRTs, significantly greater ($P < 0.05$) than inlet loads at all three HRTs. Unlike N_2O production, CH_4 production was greatest at the 8 and 16 h HRTs. Methane production was significantly less ($P < 0.05$) from 2 h HRT ($0.51 \text{ g C m}^3 \text{ day}^{-1}$) compared to 8 ($1.50 \text{ g C m}^3 \text{ day}^{-1}$) and 16 ($1.69 \text{ g C m}^3 \text{ day}^{-1}$) hour HRTs (Table 2). Methane production changed through time ($P < 0.05$), appearing to decrease from the beginning to the end of the study (Fig. 2). Methane production may continue to be reduced as the woodchips of the bioreactor age losing labile carbon and microbial activity. Dissolved CH_4 represented between 84 and 99% of total CH_4 load from the bioreactors, but surface fluxes were measurable and contributed to total mass loads.

Surface fluxes of CH_4 are not typical from Midwestern soils but may occur in wet years (Chan and Parkin, 2001; Venterea et al., 2005). The majority of CH_4 contribution from agriculture is from livestock operations (Smith and Bustamante, 2014). However, CH_4 fluxes from bioreactor surfaces averaged 0.18 (2hr HRT), 0.31 (8hr HRT), and 0.86 (16hr HRT) $\text{g CH}_4\text{-C day}^{-1}$ (Table 2). Cumulative CH_4 surface emissions between HRT treatments were not significantly different from one another ($P = 0.23$). Methane fluxes from our study were greater than emissions measured from the surface of an uncovered bioreactor in Warneke et al. (2011). The majority of bioreactor greenhouse gas emission studies have focused on N_2O emissions. Our results highlight the importance of including CH_4 measurements from bioreactor surfaces in future studies.

Dissolved mass loads were greatest in the 8 ($9.83 \text{ g CH}_4\text{-C day}^{-1}$) and 16 ($10.43 \text{ g CH}_4\text{-C day}^{-1}$) hour HRTs. Mass loads from 8 to 16 h HRTs

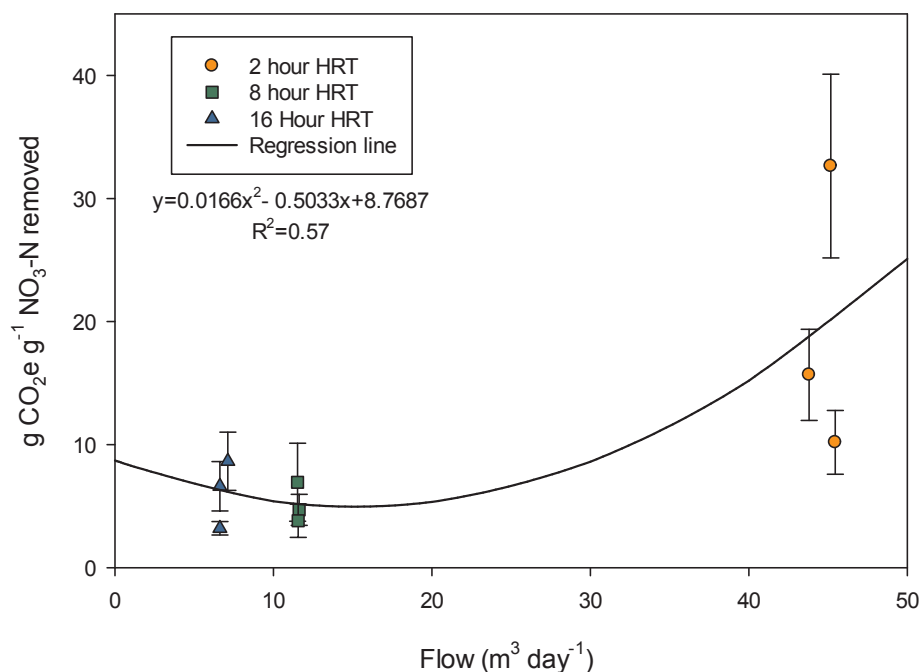


Fig. 7. Ratios of CO₂ equivalents (CO₂e) to nitrate removed for all nine bioreactors with quadratic regression line. Ratios were least from 6 to 8 h hydraulic residence times HRTs, minimizing greenhouse gas emissions and maximizing nitrate removal.

were significantly greater ($P < 0.05$) than 2 h HRT (4.60 g CH₄-C day). Methane production rates were greatest between well A and well B (Fig. 3). Dissolved CH₄ concentrations were not correlated with NO₃[−] ($r^2 = 0.02$) or dissolved O₂ concentrations ($r^2 = 0.01$). However, averaging concentrations by bioreactor position resulted in an inverse linear relationship between CH₄ and NO₃[−] ($r^2 = 0.83$) (Fig. 5). Methane production occurred without the complete consumption of NO₃[−] at all three HRTs, but greatly increased below 10 mg NO₃[−]-N L^{−1}. Unlike other field studies (Warneke et al., 2011), we found CH₄ production from the surface and dissolved in water passing through bioreactors at all three HRTs. Other studies have found CH₄ emissions from woodchip bioreactors, but typically at NO₃[−] concentrations less than 1 mg NO₃[−]-N L^{−1} (Elgood et al., 2010; Healy et al., 2012). Methane production from our bioreactors was likely from microsites within woodchips where NO₃[−] concentrations were depleted or from areas within the bioreactors where flow is not uniform.

3.4. Impacts on climate change

Nitrous oxide production within 2 h HRT bioreactors was the greatest contributor to climate change, 261 g CO₂e day^{−1} (Fig. 6). Methane represented over 80% of total emissions from 8 to 16 h HRTs and only 10% for the 2 h HRT. Healy et al. (2012) found CH₄ to be the greatest contributor across a number of bioreactor fill materials with HRTs greater than 1 day. The CH₄ contributions highlight the importance of monitoring both N₂O and CH₄ from bioreactors for accurate climate change impact estimations, particularly with HRTs greater than 16 h. Nitrous oxide produced from incomplete denitrification at 2 h HRTs could greatly increase dissolved N₂O from bioreactors and should be considered in bioreactor design. The optimal HRT minimizes impact on climate change while maximizing NO₃[−] removal. A quadratic model was used to estimate the lowest ratios of CO₂ equivalents to NO₃[−] removed. We found HRTs between 6 and 8 h removed the most NO₃[−] with the least greenhouse gas production (Fig. 7). The ratio increased at Lower and Higher HRTs, with greater N₂O production at lower HRTs and greater CH₄ production at higher HRTs. Designing field bioreactors to maintain HRTs can be difficult due to variations in drain tile flow. However, estimating a minimum HRT for maximum flow from a drain

tile is feasible. If a minimum HRT of 6 h was used in bioreactor design impacts to climate change could be minimized. Field bioreactors often exhibit low flows with HRTs much greater than 16 h. Additional research on HRTs greater than 16 h could be used to better estimate a maximum HRT recommendation.

4. Conclusions and management implications

The 2 h HRT was the greatest contributor to N₂O emissions, resulting in the greatest impact on climate change. While more CH₄ was produced in 8 and 16 h HRTs, loads did not equate to a greater impact on climate change compared to 2 h HRTs. The greatest NO₃[−] load removal in this study was observed in 2 h HRT bioreactors, but many studies have shown increased cumulative mass NO₃[−] removal from bioreactors with greater HRTs (Addy et al., 2016). The 2 h HRT was the least efficient at NO₃[−] removal, but removed the greatest mass of NO₃[−] because it received the most flow. Our results support Healy et al. (2012) and Warneke et al. (2011), concluding bioreactors produce both N₂O and CH₄ and HRT can be used in management strategies to limit greenhouse gas production from woodchip bioreactors. To meet the USDA-NRCS conservation practice standard criteria, denitrifying bioreactors are designed to treat at least 15 percent of peak flow from the drainage system and have a minimum HRT of 3 h at peak flow (Natural Resources Conservation Service, 2015). In this study and Bock et al. (2018) higher HRTs resulted in more complete denitrification and less impact on a CO₂e basis. Both studies also highlight the potential for increased N₂O production at a 3 h HRT. Changing the conservation standard to a minimum HRT of 6 h could potentially improve NO₃[−] removal and reduce N₂O losses. However, an increase in the minimum HRT would increase the maximum HRT in low flow conditions. Other environmental tradeoffs should be considered and studied in these extreme low flow conditions, including methyl mercury production in bioreactors exhibiting sulfate reducing conditions (Shih et al., 2011). The literature of greenhouse gas emissions from bioreactors is growing along with management recommendations, but a majority of the study sites are conducted on bioreactors with fresh woodchips. Greenhouse gas emissions from bioreactors will likely change with bioreactor age as NO₃[−] removal efficiency and woodchip composition change.

Consequently, HRT recommendations may change with time to maximize NO_3^- removal and minimize greenhouse gas production.

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